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Discovery

A convenient digestion method coupled to voltammetry method for simultaneous determination As(III)- Se(IV) in shrimp tissue

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ABSTRACT

Shrimp tissue (*penaeus semisulcatus*) was analyzed for simultaneous determination arsenic(III)-selenium(IV) by differential pulse cathodic stripping voltammetry, after digestion methods by various materials. Wet digestion methods with concentrated acids and alkaline treatments tested. Different approaches in digestion protocols were assessed. Best results were obtained by digesting shrimp tissue with nitric acid/ perchloric acid/sulfuric acid mixture followed by solid phase (SP) purification of the digested material. In order to quantitative extraction from shrimp muscle of the target analyte, with short times, high sensitivity and avoiding organic residues eventually affecting electrochemical measurements. Finally, the method has been validated by analyzing certified reference

material, DORM-2 (dogfish muscle). Recoveries other digestion methods were low, apparently because of incomplete destruction of organic matter and losses caused by volatilization of selenium and arsenic.

Keywords: penaeus semisulcatus, digestion, selenium, arsenic, voltammetry

1. INTRODUCTION

Toxic and persistent substances in the environment continuously increase to owing anthropic activities (Melucci et al., 2006). In fact, elements such as arsenic and selenium tend to concentrate in all aquatic matrices in the environment (suspended matter, sediment and biota), resulting in a definite presence in the aquatic food chain, which is dangerous for humans, as a consequence of the consumption of marine products. Techniques AAS, AES-ICP, X-ray fluorescence, neutron activation analysis are employed in real matrices for analysis of metals. These techniques need enrichment steps and often are in favor of selectivity or sensitivity, but in voltammetric methods a single potential in an appropriate supporting electrolyte displays qualitatively, quantitatively, selectively and simultaneously several species by employing a simple and inexpensive instrumentation. Therefore voltammetric techniques are valid and effectual option in multi component analysis of metals (Locatelli & Torsi, 2001).

The importance of selenium in environmental research is related to the fact that this element shows only a marginal range between the nutritious requirement (as an essential element) and toxic effects upon exposure (Lenz & Gmerek, 2006), in fact selenium is an essential nutrient at trace level, but toxic in excess (Sandie & Samuel, 1998). Arsenic is an important element in environmental system that majority of its forms and compounds are very toxic and carcinogenic (Piech & Kubiak, 2007).

Frequently these elements are simultaneously present in several matrices, but such presence can cause interference and effects on electrochemical behavior of both elements (Locatelli & Torsi, 2001). The CSV methods for determination Se and As are highly sensitive, but these elements very susceptible to matrix effects and other interferences (Adeloju et al., 1984). This study was focused on shrimp (*penaeus semisulcatus*) tissue that had captured from Persian Gulf, because of this species has wide diffusion in the human nutrition. When measuring trace elements in seafood products, a prior accurate and reliable sample pre-treatment step is essential. Therefore extremely thorough digestion methods are applied. Digestion methods that are suitable for analysis trace element by atomic absorption in which the digest is atomized may not be suitable for voltammetric analysis element in solution. There are not insoluble substances in fish muscle. So wet or dry decomposition treatment can be successfully applied. Dry ashing is not generally recommended because of the loss of many volatile elements.

In this work, different digestion methods, in particular several methods reported by AAS or electrochemistry (Türkmen, & Ciminli, 2007; Dugo, La Pera et al., 2006; Uluozlu et al., 2007; Dural et al., 2007; Sivaperumal et al., 2007) about the digestion of various biological samples were compared for their suitability to digest shrimp tissue in order to the simultaneous determination As(III) and Se(IV) by DPCSV. Also solid phase (SP) method apply to remove organic residues (i.e. lipids) deriving from the fat tissues, because these materials maybe interfere in measurement elements. Finally analytical parameters (linearity, sensitivity, reproducibility and, matrix effect) of the assay were evaluated and the method has been validated against certified reference material (DORM-2 dogfish muscle).

2. MATERIALS AND METHODS

2.1. Apparatus

Voltammetric measurement was carried out with a Polarograph (Metrohm 797). A hanging mercury drop electrode as the working electrode, Ag/AgCl/3M KCl double-junction as the reference electrode and a platinum electrode were used as the auxiliary electrode. The solutions were deaerated with pure argon for 5 min prior to the measurements, while argon blanket was maintained above the solution during the analysis. The solutions were deaerated for 2 min after each standard addition. A built–in motor–driven stirrer drives a PTFE stirring to stir the sample during purge and deposition.

2.2. Reagents

All reagents used were of the highest purity available (all from Merck Germany). All the solutions were prepared by deionized water. The stock solution of As(III) and Se(IV) were prepared by diluting As(III) and Se(IV) standard solutions. Cu(II) stock solution was prepared by dissolving $CuCl_2$ (1 gr) in deionized water. The voltammetric cell was rinsed every day by concentrated HCl to minimize potential contamination.

2.3. Analytical Procedure

As(III)-Se(IV) was determined by differential pulse cathodic stripping voltammetry. The application of the techniques to the simultaneous determination of As(III)-Se(IV) requires the use of HCl (as supporting electrolyte) in the presence of Cu(II). It is accepted generally that chloride ions are known to stabilize certain metal complexes at the electrode surface (Lang, & Van den Berg, 2000), probably through stabilization of the Cu(I) formed at the HMDE by complex formation with chloride (Adelaide, & Barros, 2002). In the optimization of the chemical conditions aliquots of the reagents or standards were added to the blank in the cell and a purge of 2 min was used after each addition. In the optimization of the instrumental conditions, such as potential and time of deposition, in the pre-concentration step, only 30 sec of purge was applied after varying the parameter, each scan was repeated five times to ensure that the peak current was constant. In this work an appropriate amount standard or sample was added to the analysis vessel, followed by addition of 12 M HCl and 1000 µg ml^{-1} Cu(II) to provide 0.2 M HCl and 0.07×10^{-3} M (8.8 µg ml^{-1}) Cu(II). Differential pulse cathodic stripping voltammetry (DPCSV) was performed using a deposition potential of -0.35 V versus Ag/AgCl reference electrode, applied for 200 sec with stirring, during which time As(III), Se(IV) was deposited as intermetallic compound ($Cu_xSe_yAs_z$) on the hanging mercury drop electrode (HMDE) (He, Zheng, Ramnaraine, & Locke, 2004). Stirring and purge were stopped. After a 26 sec (equilibration time), the stripping potential was scanned from -0.3 to -0.9 V versus Ag/AgCl with a voltage step 0.005 V, voltage step time 0.100 sec, scan rate 0.080 V/sec, pulse amplitude 0.080 V and pulse time 0.040 sec and the current was recorded.

2.4. Sample preparation

Wet acid digestion with nitric acid, nitric acid/hydrochloric acid, nitric acid/sulfuric acid, nitric acid/perchloric acid, hydrogen peroxide/sulfuric acid, hydrogen peroxide/ hydrochloric acid and nitric acid/perchloric acid/sulfuric acid, have been applied to shrimp muscle (fresh and dried). In order to avoid any residue of organic matter in the matrix, the digested sample was filtered on a carbon column. Carbon column was previously activated by 2.0ml of methanol followed by 2.0 ml of ultra-pure water (Dugo et al., 2006).

Wet acid digestion methods (performed into a closed vessel) on shrimp samples (were captured from Persian Gulf) were evaluated:

2.4.1. HNO_3

Transfer 5.0 g of wet or freeze dried sample to a flask or beaker. In a hood, add 5 ml conc. HNO_3 and cover with a watch glass to minimize contamination. Boiling chips may be added to aid boiling and minimize spatter. Bring to a slow boil and evaporate on a hot plate, before precipitation occurs. Continue heating and adding conc. HNO_3 as necessary until digestion is complete as shown by a light colored and clear solution. Do not let sample dry during digestion. Wash down flask or beaker walls and watch glass cover with deionized water and then filter if necessary. Transfer filtrate to a 100 ml volumetric flask, dilute to mark.

2.4.2. HNO_3/ HCl

In a hood, at 5.0 g of wet or freeze dried sample were added 2 ml HNO_3 (1:1) and 10 ml HCl (1:1), cover with a watch glass. Place flask or beaker on a hot plate and cautiously evaporate. Heat on a stream bath or hot plate until volume reduced, making certain that sample does not boil. Cool, and filter or alternatively centrifuge or let settle overnight. Quantitatively transfer sample to volumetric flask, adjust volume to 100 ml, and mix.

2.4.3. HNO_3/ H_2SO_4

At 5.0 g wet or freeze dried sample were added 5 ml conc. HNO_3 (in a hood), cover with a watch glass. Bring to slow boil on hot plate. Add 5 ml conc. HNO_3 and 10 ml conc. H_2SO_4. Evaporate on a hot plate until dense white fumes of SO_3 just appear. If solution does not clear, add 10 ml conc. HNO_3 and repeat evaporation to fumes of SO_3. Heat until solution is clear and no brownish fumes are evident (remove all HNO_3). Do not let sample dry during digestion. Cool and dilute to about 50 ml with water. Heat, to dissolve slowly soluble salts. Filter if necessary, transfer filtrate to a 100 ml volumetric flask, dilute to mark.

2.4.4. HNO_3/ $HClO_4$

In a hood, at 5.0 g freeze dried sample were added 10 ml each of conc. HNO_3 and $HClO_4$. Evaporate gently on a hot plate until dense white fumes of $HClO_4$ just appear. If solution is not clear, keep solution just boiling until it clears. If necessary, add 10 ml conc. HNO_3 to complete digestion. Cool, dilute to about 50 ml with water, and boil to expel any chlorine or oxides of nitrogen. Filter, transfer filtrate to a 100 ml volumetric flask, dilute to mark.

2.4.5. H_2SO_4/ H_2O_2

At 5.0 g wet or freeze dried sample were added 5 or 10 ml conc. H_2SO_4 and the mixture heated to 200 °C. When any water present has evaporated and the mixture taken on a brown color, 1 ml H_2O_2 30% is added. After the reaction has finished and the digestion solution turned brown again, another 1 ml H_2O_2 30% is added. The mixture is now heated up to 350-400 °C. The addition of H_2O_2 has to be repeated at this temperature until the digestion solution stays clear and colorless at the boiling point of sulfuric acid. Typically, a total of up to 5 ml hydrogen peroxide solution is needed to achieve complete digestion. After cooling down, the digestion flask is made up to 100 ml with ultrapure water.

2.4.6. Nitric acid/ perchloric acid/ sulfuric acid

at 10 g wet or freeze dried sample were added 10 ml of acid mixture nitric, sulfuric, and perchloric acid in a volume ratio of 3:1:1. This solution is efficient for complete dissolution. The samples are heated until the nitric acid boils off and perchloric acid fumes begin to appear. Heating continues until the perchloric acid boils off and SO3 fumes appear. There is little danger of perchloric acid explosions as long as sufficient nitric acid is present to decompose the bulk of the organic matter, and as long as sulfuric acid remains after the perchloric acid has evaporated to prevent the sample becoming dry. Perchloric acid should never be added directly to an organic sample.

Table 1

Analytical characteristics for the determination of As(III)-Se(IV) by differential pulse cathodic stripping voltammetry (DPCSV)
i_p = peak current (µA) , C = concentration or the electroactive species (µg. ml^{-1}), Number of independent trials = 5, r = correlation coefficent, S_r = mean standard residual deviation, D.L. = limit of detection

Element	Calibration functions of elements
As(III)	i_p= (-1.1455± 0.017) +(-2.6895)×c r^2 = 0.9990 , S_r = 1.47% , D.L. = 0.2×10^{-4} µg. ml^{-1}, Linearity = 0.39×10^{-4}- 0.40 µg. ml^{-1}
Se(IV)	i_p= (-1.0105± 0.021) + (-1.1538) ×c r^2 = 0.9975, S_r = 1.64% , D.L. = 0.11×10^{-4} µg. ml^{-1}, Linearity = 0.25×10^{-4}– 0.40 µg. ml^{-1}

Table 2

Properties of the digests after different methods of digestion

Reagents in digestion methods	Purification	Clear digested solution?	Colorless digested solution?	Elements detected in digested samples by DPCSV
HNO_3	No	No	No	No
	Carbon column	Yes	Yes	No
HNO_3/HCl	No	No	No	No
	Carbon column	Yes	Yes	No (As) / Yes (Se)
HNO_3/ H_2SO_4	No	No	No	No
	Carbon column	Yes	Yes	Yes
HNO_3/ $HClO_4$	No	No	No	No
	Carbon column	Yes	Yes	Yes
H_2SO_4/ H_2O_2	No	No	No	No
	Carbon column	Yes	Yes	No (As) / Yes (Se)
HNO_3/$HClO_4$/ H_2SO_4	No	No	No	No
	Carbon column	Yes	Yes	Yes

Table 3

Results of analysis of DORM-2 (dogfish muscle) for simultaneous determination As(III)-Se(IV) by DPCSV, using various digestion methods followed by followed by carbon column purification and comparison with the certificated value

Digestion methods	As(III) (µg.g^{-1})	Se(IV) (µg.g^{-1})
HNO_3/HCl	---	0.640 ± 0.03
HNO_3/ H_2SO_4	10.6 ± 0.94	0.91 ± 0.05
HNO_3/ $HClO_4$	12.4 ± 0.87	1.06 ± 0.04
H_2SO_4/ H_2O_2	---	0.97 ± 0.02
HNO_3/ $HClO_4$/ H_2SO_4	17.3 ± 0.98	1.38 ± 0.04
Certified value	18.0 ± 1.1	1.40 ± 0.09

3. RESULTS AND DISCUSSION

Analytical characteristics of the proposed method listed in Table 1. The linearity of the method was assessed by standard solutions of metals. The limit of detection (LOD), defined as $C_L=3S_B/m$ (where C_L, S_B and m are the detection limit, standard deviation of the blank and slope of the calibration graph, respectively). The quantitative analysis was done by standard addition method. Table 2 summarizes some properties of the digests that were obtained using each of the digestion methods evaluated. Each of the digestion techniques followed by solid phase extraction (carbon column) produced a clear digest. However, all of these digests were not suitable for As(III) and Se(IV) voltammetric analysis. For example, the HNO_3/HCl digestion method produced a clear solution that was not suitable for voltammetric analysis As(III), since As(III) is volatile and in presence hydrochloric acid is more volatile as a trichloride. Also in H_2SO_4/ H_2O_2 digestion method As(III) loss is attributable to volatilization of chlorides. In sample digested by HNO_3, arsenic and selenium were unable to be determined, in the other hand, no peak current was observed in digestion sample, even when these elements were added to the digest. It is concluded that this digestion method had also failed because of incomplete destruction organic matter. Therefore, the production of clear and colorless digest is not enough sufficient to produce a suitable digest for sample analysis by DPCSV.

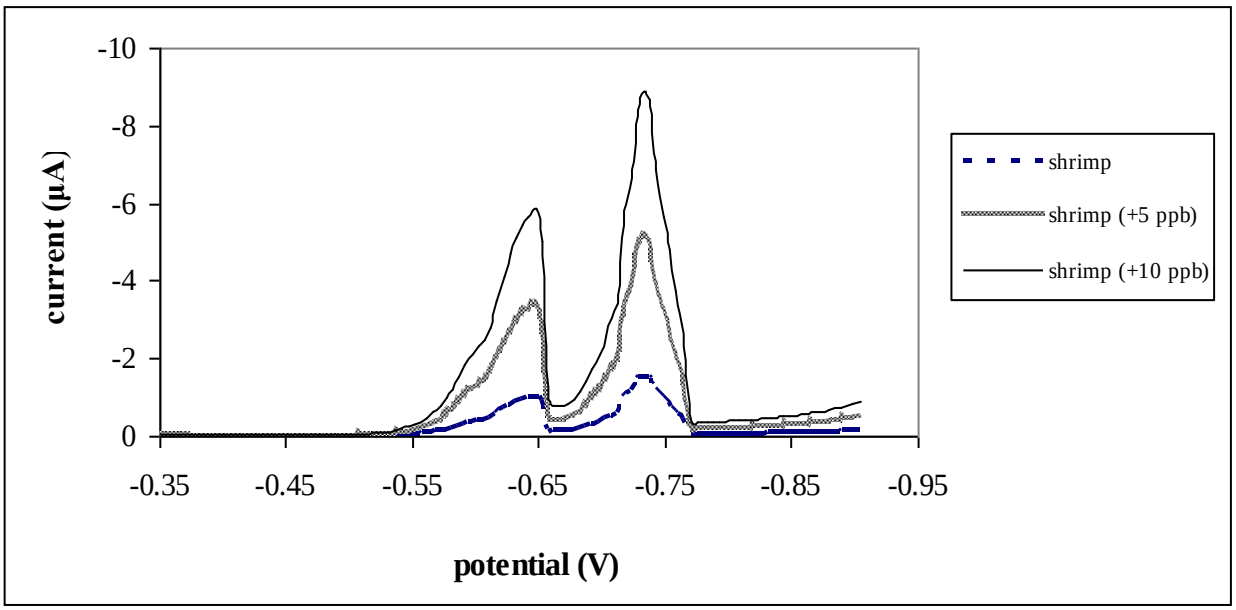

Figure 1

Obtained voltammogram for simultaneous determination AS(III)-Se(IV) by DPCSV in shrimp sample (*penaeus semisulcatus*), on HMDE electrode, after applied digestion method procedure: optimized HNO_3/ $HClO_4$/ H_2SO_4 followed by carbon column purification. The experimental conditions were: deposition potential -0.35 V for 200 sec, equilibration time 26 sec, the stripping potential was scanned from -0.3 to -0.9 V with a voltage step 0.005 V for 0.100 sec, scan rate 0.080 V/sec, pulse amplitude 0.080 V and pulse time 0.040 sec.

Table 4

Obtained accuracy for simultaneous determination As(III)-Se(IV) by DPCSV, in spiked shrimp muscle after digestion by HNO_3/ $HClO_4$/ H_2SO_4 followed by carbon column purification

Element	Element Conc. ($\mu g.g^{-1}$)	Element added ($\mu g.g^{-1}$)	Element expected ($\mu g.g^{-1}$)	Element found ($\mu g.g^{-1}$)	Accuracy [a] (%relative error)
As(III)	0.31 ± 1.10	1.20	1.51	1.22 ± 0.830	-0.192
Se(IV)	3.24 ± 0.920	1.20	4.44	4.27 ± 1.21	-3.83

[a] Accuracy = [(found- expected)/ expected] × 100

Each of digestion methods (HNO_3/ H_2SO_4, HNO_3/ $HClO_4$ and HNO_3/ $HClO_4$/ H_2SO_4) produced digestion solutions in which As(III)–Se(IV) was detected by DPCSV. In digestion methods with HNO_3/ H_2SO_4 and HNO_3/ $HClO_4$, obtained signal of elements, is not clear separation, but in digestion method with HNO_3/ $HClO_4$/ H_2SO_4, signals is completely separated, with no matrix effect (Figure 1).

The results of these methods compares in Table 3. The HNO_3/ $HClO_4$/ H_2SO_4 method produced results compatible with the certified value, with relatively low variance and maximum recovery (>96%) for arsenic and selenium. The other methods produced lower values with greater variability. This appears to be due losses of elements during digestion process and incomplete destruction organic matter. For these reasons HNO_3/ $HClO_4$/ H_2SO_4 digestion was used in further analyses. Very good agreement between the certified value and the determination obtained using HNO_3/ $HClO_4$/ H_2SO_4 method, provides a validation of the reported optimized procedure for this particular matrix.

For evaluation relative error, the analytical procedure was applied to shrimp muscle (spiked or not). The estimated element content is recorded in Table 4, relative error for both elements is <5%. Reproducibility, calculated by three determination on the same sample, and expressed as coefficient of variation (%CV), was found to be always <12 %CV.

4. CONCLUSION

Simultaneous determination AS(III)-Se(IV) by DPSCV in shrimp tissue, requires a digestion method that is extremely decomposing organic matter. Different wet digestion methods were applied. Among the methods tested, best results were obtained by digesting shrimp tissue with nitric acid/ perchloric acid/ sulfuric acid mixture followed by solid phase extraction (carbon column). With this digestion treatment and purification no matrix effect was observed and peak of elements (arsenic and selenium) were completely separated. The other digestion methods were produced digests that were unsuitable for DPSCV analysis on HMDE. Validation of the method was performed with certified reference material, DORM-2 (dogfish muscle). This fast, simple, sensitive and cheap procedure can be foreseen for other food tissue matrices.

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